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(71) Applicant *(for all designated States except US)*: CELSIS INTERNATIONAL PLC [GB/GB]; Science Park, Milton Road, Cambridge CB4 4FX (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: FOOTE, Nicholas, Peter, Martin [GB/GB]; 15 The Woodlands, Linton, Cambridge CB1 6UF (GB). GRANT, Peter, Leonard [GB/GB]; 28 Russett Avenue, Needingworth, Cambridgeshire PE17 3UE (GB).

(74) Agent: GILL JENNINGS & EVERY; Broadgate House, 7 Eldon Street, London EC2M 7LH (GB).

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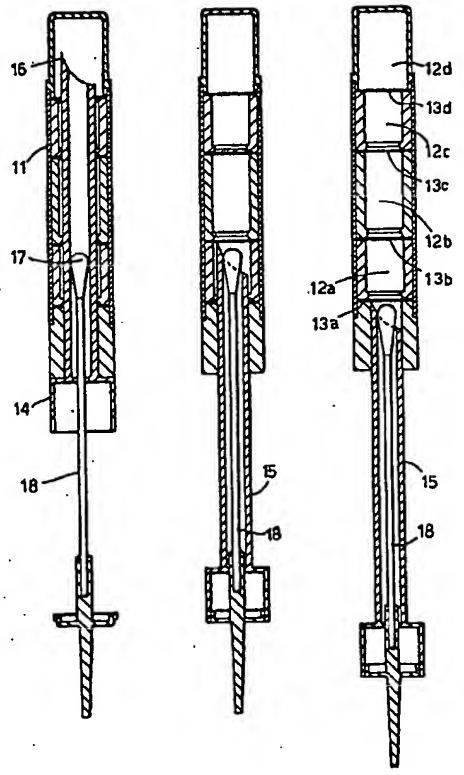
(54) Title: SAMPLE COLLECTING AND ASSAY DEVICE

(57) Abstract

An assay device comprising: a tube (1) having a removable top closure (2) on which is mounted an elongate member (4) associated with swab means (5) adapted to take up material to be assayed at the distal end of the elongate member; wherein the tube includes one or more frangible membranes (7) defining one or more compartments (3) each containing a compartmentalised agent (8), and the elongate member is movable, within the tube, to break the one or more membranes and bring said distal end into contact with the or each agent. Such a device can be used to assay microorganisms, by taking the microorganisms up in liquid on the swab, introducing the elongate member into the tube (1), and moving the elongate member (4) with respect to the tube, to break the one or more membranes and bring taken-up liquid and its contents into contact with the or each agent. A conventional bioluminescence assay can then be used.



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SAMPLE COLLECTING AND ASSAY DEVICE

Field of the Invention

This invention relates to an assay device, especially for use in hygiene monitoring and sterility testing.

Background of the Invention

For hygiene monitoring, it is desirable to have a rapid, cheap system that indicates the state of cleanliness of a surface. If microorganisms are taken up from a surface and retained in a container such as a cuvette, bioluminescence represents an efficient way to assay them. Devices adapted for this purpose are currently available from LAB M under the trade name HY-LiTE and from Biotrace Limited under the trade name Unilite. In the latter system, each assay requires a portion of luciferase-luciferin reagent to be dispensed by pipette into a small tube. After swabbing a surface and releasing microbial ATP by the action of an extractant, the swab is dipped into the reagent and then placed in a luminometer for measurement of the emitted light. This system is susceptible to interference, requires a separate pipetting step, and is inherently inaccurate.

The LAB M device is described in WO-A-9300994. It is more satisfactory in many ways. It comprises a tube having a removable closure, an elongate member having a distal swab, and a plastics member that can be pushed into the tube from below. In use, microorganisms taken up on the swab are released into a solution contained in a second tube. The plastics member of the first tube incorporates an arrangement for taking up a fixed volume of this liquid, and is impregnated with a microbial extractant. This member, with liquid sample attached, is forced into the first tube where the released ATP mixes with buffer solution. A further step then introduces the luciferase-luciferin reagent. However, this device is expensive, breakable and prone to leakage. Further, although dilution of the sample reduces interference from contaminants, sensitivity is reduced.

Summary of the Invention

According to the present invention, an assay device comprises:

a tube having a removable top closure on which is mounted an elongate swab, i.e. a member associated with means adapted to take up material to be assayed at the distal end of the elongate member;

wherein the tube includes one or more frangible membranes defining one or more compartments each containing a compartmentalised agent, and the elongate member is movable, within the tube, to break the one or more membranes and bring said distal end into contact with the or each agent. There may also be a compartment (preferably removable, at the base) which is empty, and intended for use in subsequent analysis.

Use of the device, in an assay for microorganisms, comprises taking the microorganisms up in liquid on an elongate member as defined above, introducing the elongate member into a tube as defined above, the tube containing extractant; and when desired, moving the elongate member with respect to the tube, to break the membrane(s) and bring taken-up liquid and its contents into contact with the agent.

The novel device is relatively simple to make and use. It does not require a liquid-tight seal between moving parts, nor does it involve pipetting.

Brief Description of the Drawings

The invention will be described by way of example only with reference to the accompanying drawings, in which Figures 1 to 4 are each schematic representations of embodiments of the invention. Figure 5 is a schematic representation of a particular aspect of the present invention.

Description of the Invention

The most practical way to construct a device of the invention is from the closure, an open-ended tube, and one or more wells each having the desired material therein, and

sealed by a frangible membrane or foil. Each sealed well provides a compartment in the device. If there are two or more compartments, the base of one may be defined by the top of another. They may be fitted, one on top of another, or constructed in a sequence which includes (i) introducing the desired material into a given well before (ii) sealing it with a frangible membrane, (iii) adding walls defining the next well, (iv) introducing the next desired material, etc. Of course, a combination of wells and base may be used to complete steps (ii) and (iii) together. The sealed compartment, or sequence thereof, may then be fitted to the bottom end of the tube. Liquid-tight engagement, e.g. by means of a force-fit, is appropriate. The device can thus be provided to the customer ready-constructed, or in "kit" form, with instructions as to the order in which the compartments should be arranged (for serial passage of the swab if there are two or more compartments).

In its simplest embodiment, the swab may comprise a piece of any suitable absorbent material arranged at the distal end of the elongate member. Pressing down may be sufficient to cause the member or the swab to break the or each frangible membrane. Preferably, however, the elongate member is of a resilient material having a pointed end. The elongate member may be generally tubular, a suitable absorbent material being held within the tube at its pointed distal end. The tube may be associated with means for drawing liquid up, by suction.

By way of example, the elongate member may comprise a hollow tube with an angular cut at one end, within which the swab is contained when piercing a foil barrier. This is desirable, if the swab member is not sufficiently rigid to allow satisfactory penetration through a foil barrier. Also, the angular cut gives clean piercing of a foil barrier, but leaves the pierced portion still attached to the main portion of the membrane and not free to interfere, physically or chemically, with subsequent actions.

The hollow tube may have a closed, tapering end, with a number of holes within it (rather like a sieve). This is designed to enable the swab end to be pushed into the tapered part and thus squeezed, to release any absorbed liquids.

Prior to operation, it is intended that the swab is maintained in a dry state within the device. Mechanical action will allow piercing/penetration of a first foil barrier and transfer the swab into a first compartment which contains a wetting solution. The swab is now ready to be removed in order to swab the test surface. In cases where a dry swab is required, then the swab can be removed prior to this first action. Upon returning the swab to the device, mechanical action will allow piercing/penetration of a second foil barrier and transfer of the swab into a second compartment, containing liquid reagent. After a short delay, e.g. of a few seconds, a further mechanical action will allow piercing/penetration of a third foil barrier and transfer of the swab into a third compartment, containing a freeze-dried reagent. The liquids from the preceding compartments will also enter the third compartment and reconstitute the freeze-dried reagent, producing bioluminescence or alternative reaction to detect ATP.

Fig. 1 shows a relatively simple embodiment of the invention. The illustrated device comprises a tube 1, an associated cap 2 and a well member 3 that is a force-fit in the base of the tube 1. To the cap 2 is attached a swab member comprising an elongate member 4 having at its distal end an absorbent material 5 that will take up liquid. The cap (or "plunger") has two positions. In the up position (as illustrated), it is in contact with extractant 6 (which is also a reconstitution buffer for the agent). The cap 2 and swab 4, 5 can be removed, used to swab a surface, and then replaced. When lowered, to the down position, a foil partition 7 sealing the wall 4 is broken, a freeze-dried reagent 8 is automatically reconstituted and mixed with the

extractant/sample. The down position may be achieved by straight pushing or by twisting/screwing down. The whole tube is then placed in a custom-built luminometer.

Fig. 2 shows a device suitable for use in testing water samples, with many of the same components as the device shown in Fig. 1. However, the swab illustrated in Fig. 1 is replaced by a syringe member having a tubular sheath 9 and a plunger head 10; any similar liquid sampling device may be used (fixed or variable volume). If a swab extract needs to be diluted because of cleaning agent residues etc, the two methods are combined. In this case, the swab tube does not need reagent. The swabbing is done with extractant as usual, and then the solution is sampled with the syringe device and assayed as described above.

Fig. 3 shows another variant of the same concept. In this case, the absorbent material 5 of the swab is mounted on a member 5a within a tubular member 9 having an angled piercing end 9a.

Very similar systems may be used for sterility testing of surfaces and liquids. In these cases, the freeze-dried bioluminescence reagent described above is replaced by sterile medium (either dried or liquid) which optionally may be chosen for a low ATP content, and the extractant with water or PBS. After swabbing, or taking a liquid sample with the syringe, the foil is pierced as before and the sample incubated for e.g. 24 hours.

After incubation, there are various ways of detecting the presence of microorganisms. For example, (a) a sample is taken by pipette for a normal bioluminescence assay of total ATP; (b) a sample is taken with a swab and assayed as described above with reference to Fig. 1; or (c) a sample is taken with the syringe device and assayed as described above with reference to Fig. 2. If the original sample was a liquid, the same syringe may be used for the transfer. An indicator medium may be used for a more specific sterility test, e.g. for the presence/absence of E. coli.

of course, after sufficient incubation time, the presence of growth will be observable as turbidity.

As illustrated in the drawings, the agent that is in a device of the invention may be a freeze-dried bioluminescence reagent. Alternatively, it may be water or a growth/culture medium. More generally, the agent will comprise whatever material is necessary, sufficient or appropriate for an assay to be conducted, on the material collected.

As illustrated in Figs. 1 and 2, the novel device may comprise one compartment. In addition to an agent in the compartment, liquid (e.g. for reconstitution or cell lysis) may be held above the membrane, within the tube. However, if a swab breaks up in liquid over a long storage period, it could be kept dry until the time of use by holding it separate from the swabbing solution. For this purpose, there may be three primary positions for the movable part (if it is assumed that the relatively movable parts have preferred positions; alternatively, if there is a satisfactory friction fit, the relative movement can be continuous or interrupted, as desired by the operator, manual or automatic).

Figs. 4A-4C show an embodiment of the invention having a plurality of compartments. The parts are manufactured so that they fit together snugly, so that the device can be used in any orientation.

Fig. 4 shows an outer tube 11 onto or in which fit a series of four compartments 12a, 12b, 12c, 12d. Compartment 12d defines the base (in terms of the orientation used herein for illustration; top as illustrated) of the device. The compartments are separated from the open end of the tube and from each other by respective foil barriers 13a, 13b, 13c, 13d.

The open end of the tube cooperates with a closure member 14 on which an elongate tube 15 is mounted, and which has an angled, pointed end 16 adapted to break the

foil barriers cleanly. An absorbent swab 17 is mounted on an elongate member 18 that is moveable within the tube 15.

After use of the swab to pick up a sample to be assayed, the device is moved through and between successive positions shown in Figs. 4C, 4B and 4A, in the last of which all foil barriers have been pierced. It is proposed that each of compartments 13a, 13b and 13c contain a respective agent; compartment 13d is empty. When the combination of these agents and the analyte is introduced into compartment 13d, that can be removed, for analysis.

There may thus be more than one compartment, used in sequence. For instance, further compartments could optionally contain: neutralisers of biocide or other residues; liquid or dry growth medium (non-selective or selective); or reagents for specific detection (e.g. colorimetric enzyme substrates). This last possibility may be appropriate for detection by a colour reaction rather than bioluminescence. The reaction could be the exponential amplification described in WO-A-9425619 with the two "pre-mixes" separated by a membrane, or even a sensitive but simpler linear amplification reagent.

In one embodiment of an assay device, which may be used as the elongate member described above, a swab is combined with a brush, e.g. in a combined sterile device. A particularly suitable device comprises an elongate handle and, on opposite faces of a head, the brush and the swab. Such a device may be used by first brushing the surface in the presence of a suitable sterile fluid, to abrade bio-film and release micro-organisms there and in internal angles, turning the device over and swabbing the surface to collect all the micro-organisms thereon. Alternatively, the fluid may lyse or disrupt the microorganisms, and cellular material may be collected by the swab for analysis. The swab can simply be provided as, for example, a piece of sponge-like material.

Fig. 5 illustrates a device of the type described immediately above, in which the elongate handle is hingedly

attached to the head. Fig. 5 shows an elongate handle 21 and head having a brush 22 and a swab 23 on opposite faces thereof. A simple hinge 24 is provided between the head and the handle.

Devices of the invention are simple to manufacture and use. They can be made disposable. After swabbing, the micro-organisms or collected material can be determined in conventional manner.

CLAIMS

1. An assay device comprising:

a tube having a removable top closure on which is mounted an elongate member associated with means adapted to take up material to be assayed at the distal end of the elongate member;

wherein the tube includes one or more frangible membranes defining one or more compartments each containing a compartmentalised agent, and the elongate member is movable, within the tube, to break the one or more membranes and bring said distal end into contact with the or each agent.

2. A device according to claim 1, wherein the elongate member is fixed to the closure which is movable with respect to the tube.

3. A device according to claim 1, wherein the elongate member is movable with respect to the closure.

4. A device according to any preceding claim, wherein the or an agent comprises freeze-dried luciferase and/or luciferin.

5. A device according to any preceding claim, wherein the or an agent comprises water or a growth medium.

6. A device according to any preceding claim, wherein the elongate member is tubular and has an angled end.

7. A device according to any preceding claim, wherein the tube comprises an open-ended cylindrical part and, in liquid-sealing relationship therewith, the one or more compartments.

8. A device according to claim 7, wherein the end compartment, or an additional, empty compartment, is removable, for analysis.

9. An assay for microorganisms, which comprises taking the microorganisms up in liquid on an elongate member as defined in any preceding claim, introducing the elongate member into a tube as defined in any preceding claim, and moving the elongate member with respect to the tube, to break the one or more membranes and bring taken-up liquid and its contents into contact with the or each agent.

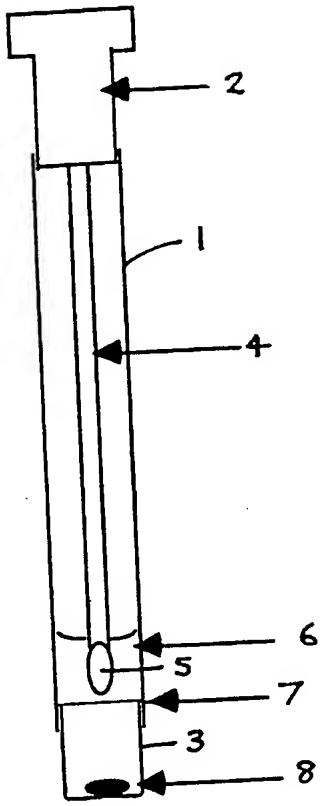

Fig. 1

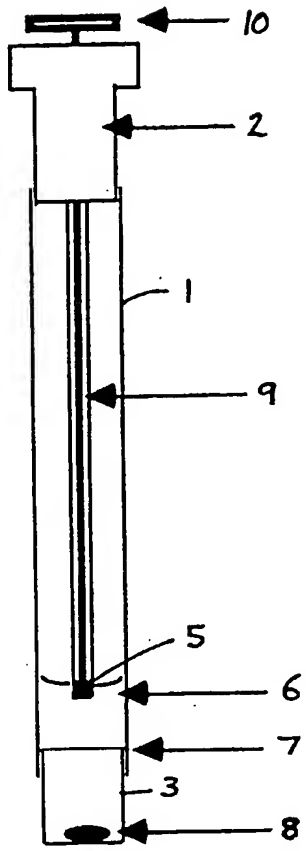

Fig. 2

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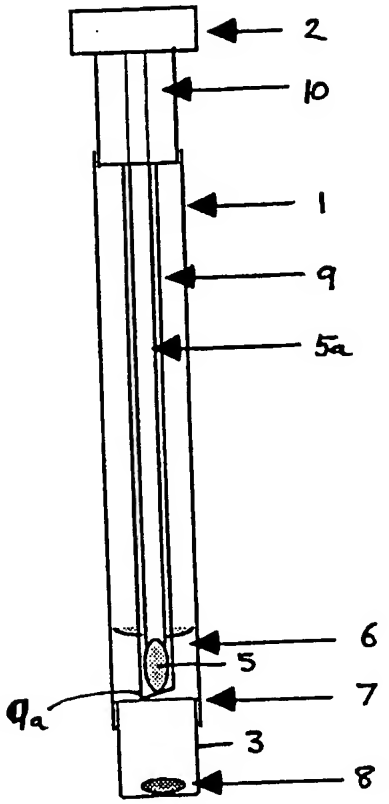

Fig. 3

Fig.4 A

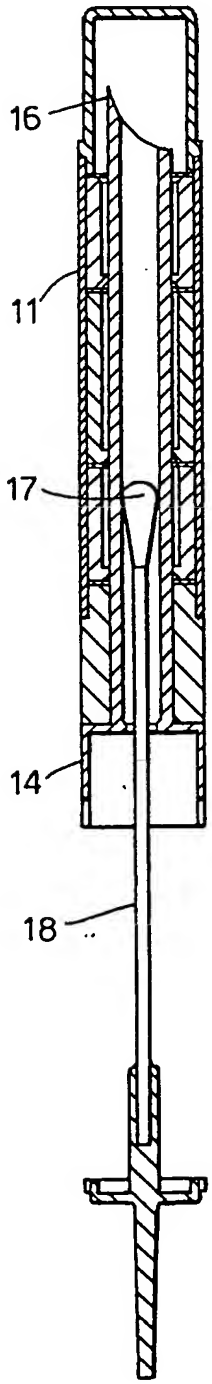

Fig.4B.

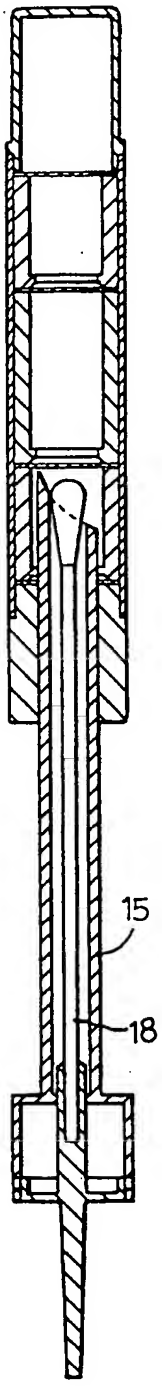

Fig.4 C.

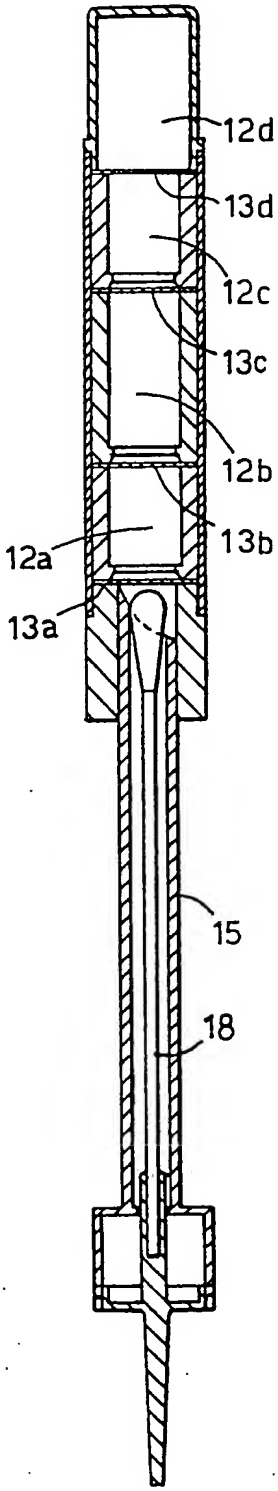

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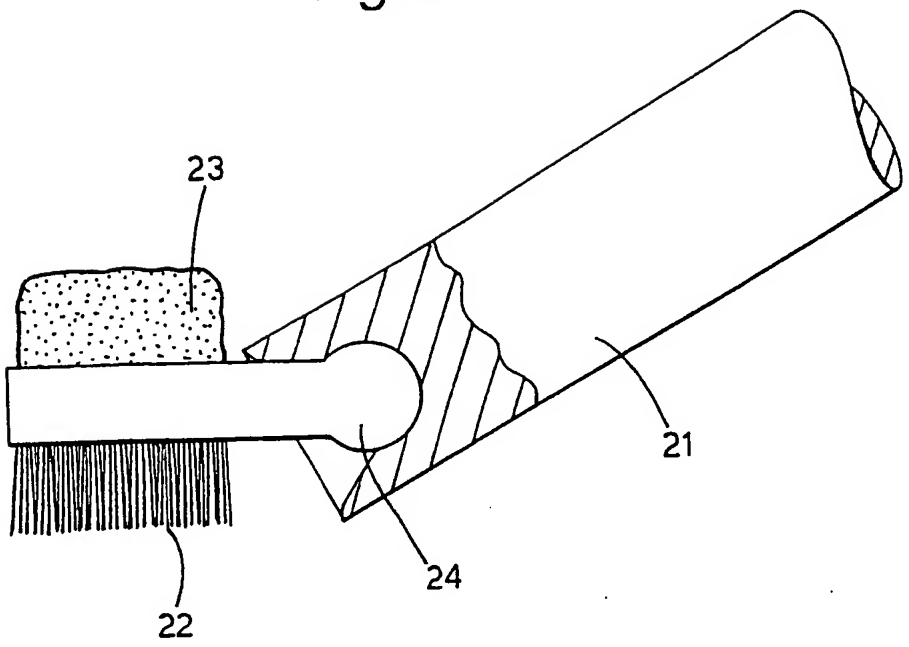
Fig.5.



INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 95/00649

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N1/02 B01L3/00 C12M1/30 G01N21/76

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IPC 6 G01N B01L C12M A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO-A-93 09431 (UNIV BIRMINGHAM) 13 May 1993	1-3,6,9
Y	see figures 1-3,6,7	4
X	US-A-4 353 868 (JOSLIN JOEL A ET AL) 12 October 1982	1,2,5,7, 8
A	see column 2 - column 3; figures	6
X	US-A-4 150 950 (TAKEGUCHI MILTON M ET AL) 24 April 1979 see column 2, line 54 - column 3, line 19; figures	1,2,5
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US-A-5 188 965 (WANNLUND JON C) 23 February 1993 see column 3, line 36 - line 41 ---	4
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1

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter nal Application No
PCT/GB 95/00649

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